

*Rearing and foraging affects bumblebee (*Bombus terrestris*) gut microbiota*

Article

Accepted Version

Newbold, L. K., Oliver, A. E., Cuthbertson, L., Walkington, S. E., Gweon, H. S. ORCID: <https://orcid.org/0000-0002-6218-6301>, Heard, M. S. and van der Gast, C. J. (2015) Rearing and foraging affects bumblebee (*Bombus terrestris*) gut microbiota. *Environmental Microbiology Reports*, 7 (4). pp. 634-641. ISSN 1758-2229 doi: <https://doi.org/10.1111/1758-2229.12299>
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To link to this article DOI: <http://dx.doi.org/10.1111/1758-2229.12299>

Publisher: Wiley-Blackwell

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1 **Rearing and foraging affects bumblebee (*Bombus terrestris*) gut microbiota**

2

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11

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13

14 Running title: Ecological effects on bumblebee gut microbiota

15

16 **Keywords:** *Bombus terrestris* / pollinators / gut microbiome / commonness and rarity / bumblebees /
17 bacterial communities

18

19 Data deposition: The sequence data reported in this paper have been deposited in the European
20 Nucleotide Archive under study accession number ERP007145, and sample accession number
21 ERS557783.

22

23 **Summary**

24 Bumblebees are ecologically and economically important as pollinators of crop and wild plants,
25 especially in temperate systems. Species, such as the buff-tailed bumblebee (*Bombus terrestris*), are
26 reared commercially to pollinate high value crops. Their highly specific gut microbiota, characterised
27 by low diversity, may affect nutrition and immunity and are likely to be important for fitness and colony
28 health. However, little is known about how environmental factors affect bacterial community structure.
29 We analyzed the gut microbiota from three groups of worker bumblebees (*B. terrestris*) from distinct
30 colonies that varied in rearing and foraging characteristics: commercially reared with restricted
31 foraging (RR); commercially reared with outside foraging (RF); and wild-caught workers (W). Contrary
32 to previous studies, which indicate that bacterial communities are highly conserved across workers,
33 we found that RF individuals had an intermediate community structure compared to RR and W types.
34 Further, this was shaped by differences in the abundances of common OTUs and the diversity of rare
35 OTUs present which we propose results from an increase in the variety of carbohydrates obtained
36 through foraging.

37

38

39 Introduction

40 Insects and other pollinators provide a vital ecosystem service to 87.5% of the world's plant species
41 (Ollerton et al., 2011) and demand for pollination services in crops is high (estimated global value of
42 €153 billion; Gallai et al., 2009). As a consequence, there is an increasing awareness of the
43 ecological and economic importance of such organisms. However, whilst demand for pollination
44 services continues to rapidly increase, there is growing evidence for declines in pollinator populations
45 (Biesmeijer et al., 2006; vanEngelsdorp et al., 2008; Aizen and Harder, 2009; Potts et al., 2010a;
46 Potts et al., 2010b). Declines are likely driven by multiple factors including disease, pesticide use,
47 host plant loss and changes in land management (Cameron et al., 2011; Dicks et al., 2013; Scheper
48 et al., 2014). A link between the reduction of plant pollination, and a drop in pollinator diversity and
49 abundance is also well established (Memmott et al., 2004; Biesmeijer et al., 2006; Albrecht et al.,
50 2012). An increasing human population will only intensify demands on wild and managed pollinator
51 populations to meet future food security needs (Klein et al., 2007; Aizen et al., 2008).

52

53 In temperate systems, eusocial bumblebees (*Bombus* spp.) are important and prolific plant
54 pollinators. Some species are commercially managed to pollinate high value glasshouse and fruit
55 crops (Klein et al., 2007; Leonhardt and Blüthgen, 2012). This practice is increasingly common, with
56 between 30,000-60,000 bumblebee colonies per year being imported into the UK alone (Lye et al.,
57 2011). Ensuring the production of healthy bumblebee colonies will be vital to sustain the growing
58 demand for their services (Pettis et al., 2012). There is therefore interest in how commercially reared
59 bees may differ from wild types in terms of physiology, and how interactions between them may affect
60 fitness (Otterstatter and Thomson, 2008).

61

62 The insect gut is known to harbour a microbial community which is thought to aid host fitness through
63 enhanced nutrition, immunity and colony health (Dillon and Dillon, 2004; Warnecke et al., 2007;
64 Cariveau et al., 2014; Pernice et al., 2014). Recent studies suggest the *Bombus* gut bacterial
65 community is predominately comprised of members from: Orbaceae (Gammaproteobacteria),
66 Lactobacillaceae (Firmicutes), Neisseriaceae (Betaproteobacteria), Acetobacteraceae
67 (Alphaproteobacteria), Bacteroidetes and Actinobacteria (Koch and Schmid-Hempel, 2012; Koch et
68 al., 2013; Kwong and Moran, 2013; Cariveau et al., 2014). While much of the evidence suggests that

69 the gut microbiota of bumblebees are highly conserved and of relatively low diversity (Koch and
70 Schmid-Hempel, 2011b; Martinson et al., 2011) it has been shown that detectable shifts in bumblebee
71 gut bacterial diversity may occur in response to infection (Koch et al., 2012; Cariveau et al., 2014).
72 How other environmental changes affect gut microbial community structure remains unexplored.

73

74 Here, we utilized 16S rRNA gene targeted next generation sequencing techniques to analyze the gut
75 microbiota from three groups of individual adult female bumblebees (*Bombus terrestris*) from distinct
76 colonies that were: commercially reared with no outside (restricted) foraging (RR, $n = 6$), commercially
77 reared but released for outside foraging (RF, $n = 10$) and field-caught workers collected from
78 Buckinghamshire and the Isle of Wight, UK (W, $n = 7$). Given the low diversity and highly specific
79 microbiota reported previously, we adopted a null hypothesis that diversity and composition of *B. t.*
80 *audax* host gut microbiota would not be influenced by rearing and foraging conditions. The current
81 study aimed to establish whether gut microbiota responded to host foraging, i.e. does a commercially
82 reared host, with controlled food resources (within colony standardised pollen and nectar solution)
83 have a detectably different gut microbiota from that of wild populations.

84

85

86

87 **Results and discussion**

88 Bacterial diversity and composition from whole gut samples was assessed using 16S rRNA gene
89 targeted high-throughput sequencing. From 23 bee gut samples, a total of 2,465,708 sequence reads
90 (mean \pm SD per sample, 107204.7 ± 59212.6) were included in the final analysis and 373 distinct
91 operational taxonomic units (OTUs) identified. The average numbers of bacterial sequence reads per
92 sample were similar among the three groups: commercially reared but restricted to colony (RR),
93 $96,484 \pm 55,741$ ($n = 6$); commercially reared but with outside foraging (RF), $100,533 \pm 53,812$ ($n =$
94 10); and wild-caught workers (W), $125,924 \pm 64,867$ ($n = 7$). The number of OTUs we identified is
95 higher than that in studies applying traditional culture independent techniques - ranging from 9 to 146
96 sequenced OTUs (Koch and Schmid-Hempel, 2011b; Martinson et al., 2011). Thus, the increased
97 sampling depth through the application of next generation sequencing (NGS) appears to have
98 captured more of the inherent gut microbial diversity. When compared to other insects guts (e.g up to
99 726 OTUs were identified in the termite hind gut alone, Köhler et al., 2012), an overarching richness
100 of 373 OTUs is relatively low, although comparable to that of the honey bee (Moran et al., 2012),
101 suggesting that the bumblebee gut microbiome does indeed represent a low diversity, specialized
102 community.

103

104 It is expected that a microbial metacommunity would display a positive relationship between
105 frequency and abundance of individual taxa (OTUs) from within its constituent communities (van der
106 Gast et al., 2011). Consistent with this prediction, the abundance of individual bacterial OTUs, across
107 all samples (Figure 1a), was significantly correlated with the number of individual gut sample
108 communities that they occupied. Separating component taxa within a host microbiota into common
109 and rare groupings reveals important aspects of taxa-abundance distributions (van der Gast et al.,
110 2011; van der Gast et al., 2014). Here, we partitioned the OTUs into 'common' (defined as those
111 present in the upper quartile of sample occupancy with $>75\%$ across all samples) and 'rare'
112 groupings. The 28 common OTUs accounted for 97.4% of the total sequence abundance while the
113 rare group comprised the majority of the diversity (345 'rare' OTUs). Similarly, Cariveau et al. (2014)
114 determined that high abundance OTUs represented 98.9% of sequences from *B. bimaculatus* and *B.*
115 *impatiens* gut microbiota samples.

116

117 Mean OTU richness in the whole microbiota was significantly higher within the RF group ($121.5 \pm$
118 10.4 , mean \pm SD) when compared to the other samples (RR, 97.2 ± 18.7 ; and W, 83.0 ± 2.1 ; Figure
119 1b and Table S1). The same significant pattern was reflected in the rare microbiota (RR, 71.2 ± 18.1 ;
120 RF, 96.8 ± 9.8 ; and W, 57.4 ± 2.0), but not in the common microbiota which did not significantly differ
121 between groups (RR, 26.0 ± 1.2 ; RF, 24.7 ± 1.11 ; and W, 25.6 ± 0.8 ; Figure 1b and Table S1). We
122 therefore assert that observed patterns in richness are driven by compositional changes in the rare
123 microbiota. This was confirmed by pair wise comparisons of turnover rates (number of taxa/OTUs
124 eliminated and replaced; Figure 1c), where whole microbiota turnover between groups followed that of
125 the rare microbiota comparisons. No turnover was observed between the common microbiota (Figure
126 1c), however the common microbiota did contribute most to patterns of whole microbiota composition
127 (Figure 1d). Bray-Curtis quantitative index similarity (S_{BC}) revealed the whole microbiota to be highly
128 similar to the corresponding common microbiota (mean $S_{BC} = 0.99 \pm 0.01$, $n = 3$ pair wise
129 comparisons). Conversely, the rare microbiota was highly dissimilar between whole microbiota and
130 corresponding rare microbiota (mean $S_{BC} = 0.04 \pm 0.03$), and were divergent between rare microbiota
131 groups (mean $S_{BC} = 0.23 \pm 0.15$; Figure 1d).

132

133 Analysis of the uniqueness and sample group allocation of OTUs (Figure 2) demonstrated that, in
134 addition to the 28 common OTUs, a further 102 OTUs (taxa) were shared across all treatments.
135 These appear to be an integral part of the wild *B. t. audax* gut microbiota, and therefore likely to be
136 retained across generations. Interestingly, when looking at the allocation of rare OTUs the reared
137 foraging group had the highest number unique of OTUs (75) when compared to the other sample
138 group types (RR = 9, W = 13). Further, none of the OTUs detected were shared solely between the
139 RR and W groups, suggesting that although gut microbiota from commercially reared populations are
140 distinct from wild populations, when allowed to forage a shift in microbiota from a commercially reared
141 to wild pattern occurs. As such the RF group would represent a population with microbiota in flux,
142 showing a pattern which shares both commercially reared and wild attributes. If this is the case it
143 would be interesting to consider whether the RF gut microbiota population would fully transition to a
144 wild type and how long such a transition would take. Analysis of similarity (ANOSIM) tests give further
145 weight to the patterns observed. While the microbiota (whole, common and rare) from RR and W

146 samples were significantly divergent (Table 1), the RF microbiota shared attributes with both the RR
147 and W groups' microbiota.

148

149 In order to determine which OTUs contributed most to the observed shift in community abundance
150 and composition similarity percentage (SIMPER) analysis was performed (Table 2). Representative
151 OTUs commonly found within insect and hymenopteran guts were prevalent within the bumblebees
152 studied here - including members of the Neisseriaceae, Orbaceae, Enterobacteriaceae,
153 Lactobacillaceae, Pseudomonadaceae and Bifidobacteriaceae (Kosako et al., 1984; Babendreier et
154 al., 2007; Novakova et al., 2009; Killer et al., 2010; Wilkes et al., 2011; Koch et al., 2013; Duron,
155 2014; Engel et al., 2014; Killer et al., 2014b; Killer et al., 2014a). Two common microbiota group
156 OTUs, identified as *Snodgrassella alvi* and *Gilliamella apicola*, contributed the most to the dissimilarity
157 between groups. Both have previously been found to be dominant members within honeybees and
158 other bumblebee species (Koch and Schmid-Hempel, 2011a; Kwong and Moran, 2013). *S. alvi* had a
159 higher relative abundance in the RR samples (52.1%) than both the RF (29.5%) and W (22.4%)
160 samples. Conversely, *G. apicola* was more abundant in the wild samples (30.9%) than the reared (RR
161 = 22.9% and RF = 17.9%).

162

163 Analysis of the genomes of these organisms has suggested that they perform complementary roles
164 within the bee gut. Kwong et al. (2014b) suggest that *G. apicola* is a saccharolytic fermenter,
165 possessing the genes for pathways associated with carbohydrate metabolism, whereas *S. alvi* shows
166 no evidence of these, instead possessing pathways involved in the metabolism of carboxylates. It
167 appears that increases in *G. apicola* mean relative abundance in the wild bees represents a biological
168 response to increased foraging (i.e, a wide range of pollen and nectar types) which contrasts with
169 commercially reared bees, fed upon a single nectar source and restricted (irradiated) pollen. This is
170 further supported by the presence of other OTUs which exhibited increases in relative abundances
171 related to foraging. The common OTU identified as *Arsenophonus nasoniae* demonstrated an
172 increase in abundance in favour of foraging ability (RR=0.02%, RF=6.1%, and W= 15.8%, Table 2). A
173 genomic study based upon *Arsenophonus nasoniae* indicated that this species contains intact
174 pathways for carbohydrate metabolism (Darby et al., 2010). A common OTU identified as
175 *Fructobacillus* also increased with foraging (RR = 0.02%, RF = 0.29%, W = 12.5%). The genus

176 *Fructobacillus* is a group of fructophilic lactic acid bacteria that prefer fructose as a growth substrate
177 and inhabit fructose-rich habitats, including bumble (Koch and Schmid-Hempel, 2011b) and honey
178 bee guts (Endo and Salminen, 2013). Interestingly, there appeared to be role differences occurring
179 within related taxa. Members of the *Lactobacillus* genus are able to metabolise multiple carbohydrate
180 types (Killer et al., 2014a; Kwong et al., 2014a); here a common OTU identified as *Lactobacillus*
181 *kunkeei* increased in relative abundance with the ability to forage, whereas another common and
182 distinct *Lactobacillus* OTU decreased (Table 2). Overall, wild foraging represents an increase in the
183 range and diversity of pollen/nectar sources and therefore the bacteria able to process these
184 additional carbohydrate types.

185

186 Finally, canonical correspondence analysis revealed that variance in microbiota was explained by
187 foraging, rearing and host weight (Table 3 and Figure S1). Undetermined variation could be explained
188 by factors not measured here, for example infection with microbial parasites (e.g. *Crithidia* and *Nosema*)
189 and colony age; both previously associated with differences in *Bombus* spp. gut communities (Koch et
190 al., 2012; Cariveau et al., 2014).

191

192 In eusocial bees common bacteria are often considered to be synonymous with indigenous/core host
193 gut microbes and are most likely acquired through vertical transmission or within colony interactions
194 (Powell et al., 2014). In contrast, rare/non-core microbiota often contain members which are
195 associated with non-host environments, and are most likely acquired through horizontal transmission
196 (Cariveau et al., 2014). Within our study the rare bacteria shaped observed patterns in diversity. We
197 suggest these detected changes are likely to be through low abundance organisms which have
198 changed in response to host bees foraging on more diverse food resources, in addition to the
199 horizontal acquisition of bacteria from the environment. In a recent study in honey bees it was found
200 that the majority of transmission of gut bacteria was through within hive interactions, rather than
201 environmental exposure (Powell et al., 2014). If this pattern holds true for bumblebees it would
202 suggest that, although the environment does undoubtedly serve as an important and variable
203 reservoir for bacterial immigration, the existing gut microbiota has the capacity to adapt to new
204 foraging resources.

205

206 Overall, we have shown that significant variation in microbiota can result from intraspecific differences
207 in bumblebee rearing and foraging. Given the vital ecosystem services bumblebees provide in
208 pollination of crop and native plants future work should focus on the temporal and functional
209 significance of these shifts in bacterial diversity and composition, and any subsequent effect upon
210 host health and fitness.

211 **Experimental procedures**

212 *Bumblebee samples*

213 Commercially reared (Biobest N.V., Westerlo, Belgium) mature female worker individuals of *Bombus*
214 *terrestris audax* (Table S2) were collected after 26 days into the experiment from distinct colonies that
215 were restricted to colony (RR, $n = 6$) or allowed to forage (RF, $n = 10$) in agricultural land near to the
216 NERC Centre for Ecology & Hydrology, Wallingford, Oxfordshire, UK. Wild female worker individuals
217 (W, $n = 7$) were collected in July 2009 from within agricultural landscapes on the Isle of Wight, UK (n
218 = 3), and the Hillesden Estate, Buckinghamshire, UK ($n = 4$) as part of a previous study (Carvell et al.,
219 2012). Molecular microsatellite analysis data were examined, generated from a previous study
220 (Carvell et al., 2012), to minimise probability of processing collected individuals from the same colony.
221 Members of the reared restricted (RR) group were reared within laboratory conditions with a diet
222 consisting of 'Biogluc' (a 66% commercial sugar solution) and fresh (frozen), gamma irradiated pollen,
223 both supplied by Biobest N.V, Belgium. Members of the reared foraged group (RF) were treated
224 identically to the lab reared group until introduction to the wild. At this point - in order to encourage
225 foraging from the local agricultural landscape - no additional nutritional substitute was provided. RR
226 and RF individuals were sampled during July and August 2013.

227

228 *DNA extraction and sequencing*

229 Whole guts from individual specimens (frozen at -80°C within 2 hours of collection) which had been
230 commercially reared or captured in the wild, were used to extract microbiome DNA using the
231 PowerSoil®-htp 96 Well Soil DNA Isolation Kit (Mobio Laboratories Inc., Carlsbad, CA), under the
232 manufacturers recommended protocol. In addition, PCR negative controls consisting of extraction
233 and PCR blanks were also processed and likely kit contaminants removed from analysis (Salter et al.,
234 2014). Approximately 20-30 ng of template DNA was amplified using Q5® high-fidelity DNA
235 polymerase (New England Biolabs, Hitchin, UK) each with a unique golay barcoded primer. After an
236 initial denaturation step at 98°C for 2 min, individual PCR reactions employed 25 cycles of an initial
237 30 sec, 98°C denaturation step, followed by annealing phase for 30 sec at 53°C , and final extension
238 step lasting 90 secs at 72°C . All reactions employed a final extension step of 5 min at 72°C . Primers
239 based upon the universal primers 27F (5'- CCATCTCATCCCTGCGTGTCTCCGACTCAG) and 338R
240 (5'- GCTGCCTCCCGTAGGAGT) were adapted to include ion torrent linker, golay barcode (Whiteley

241 et al., 2012) and spacer sequences (Table S2). An amplicon library consisting of ~400 bp amplicons
242 spanning the V1-V2 hypervariable regions of the 16S rRNA gene was generated from gel purified
243 pooled products of 4 replicate PCR reactions, per sample. Quantification was performed on an Agilent
244 2200 TapeStation system and an equimolar mix of PCR products was prepared and diluted to 20pM
245 in dH₂O. This library was sequenced using an Ion Torrent Personal Genome Machine (Life
246 Technologies, Paisley, UK) with a 316 chip.

247

248 *Sequence analysis*

249 The Mothur sequencing analysis platform was used to analyse the resulting data (Schloss et al.,
250 2009; Schloss et al., 2011). Sequence quality checks included the removal of failed reads, low-quality
251 ends, tags and primers. Further, sequences were aligned against the Mothur SILVA reference
252 bacterial database and any unaligned sequences that included ambiguous base calls and/or
253 homopolymers longer than 8 bases were also eliminated. Finally, chimeras were identified and
254 discarded through Mothur using the UCHIME algorithm (Edgar et al., 2011). The resultant alignment
255 was used to assemble operational taxonomic unit (OTU) clusters at 96% identity, through distance
256 measures (Schloss and Handelsman, 2005, 2006). Taxonomic identity of these OTUs was assigned
257 using the default settings with the mothur RDP reference database. As an additional measure the
258 identity of reference sequences from key OTUs was corroborated using the NCBI's BLASTN program.
259 OTUs identified in negative controls were removed from further analysis (Salter et al., 2014). The raw
260 sequence data reported in this study have been deposited in the European Nucleotide Archive under
261 study accession number ERP007145 and sample accession number ERS557783. The relevant
262 barcode information for each sample is shown in Table S2.

263

264 *Statistical analysis*

265 Operational taxonomic units (OTUs) were partitioned into common and rare microbiota groups using a
266 modification of the method previously described (van der Gast et al., 2011; van der Gast et al., 2014).
267 Based on a significant positive distribution-abundance relationship, the persistent and abundant
268 common OTUs were defined as those in more than 75% of all samples, while all other OTUs falling
269 outside of the upper quartile were considered to be rare. Richness (S^*) was used as previously
270 described (Rogers et al., 2013). It is known that pair wise comparisons will be affected by large

271 differences in sample size (Gihring et al., 2012). Therefore, S^* was calculated with a uniform re-
272 sample size (to match the smallest sequence size in each microbiota group [whole, common, and
273 rare]) following 1000 iterations in each instance and performed in R version 3.1.1 (Oksanen et al.,
274 2013; The R Development Core Team, 2013)

275

276 Taxa turnover between consecutive samples was measured using the method described by Brown
277 and Kodric-Brown (1977). Turnover was defined as: $t = b + c / S1 + S2$. Where b = the number of
278 OTUs present only in the first sample; c = the number of OTUs present only in the second sample; $S1$
279 = the total number of OTUs in the first sample; and $S2$ the total number of OTUs in the second
280 sample (Brown and Kodric-Brown, 1977). Two-sample t -tests, regression analysis, coefficients of
281 determination (r^2), residuals and significance (P) were calculated using Minitab software (version 16,
282 Minitab, University Park, PA, UK). The Bray-Curtis quantitative index of similarity and subsequent
283 average linkage clustering of community profiles was performed using PAST (Paleontological
284 Statistics, version 3.01) program, available from the University of Oslo
285 (<http://folk.uio.no/ohammer/past>). Analysis of similarity (ANOSIM) and similarity of percentages
286 analysis (SIMPER) were performed using the PAST (version 3.01). The Bray-Curtis quantitative index
287 of similarity was used as the underpinning community similarity measure for both ANOSIM and
288 SIMPER analyses. Canonical correspondence analysis (CCA) was used to relate the variability in the
289 distribution of microbiota between groups to environmental factors. Environmental variables that
290 significantly explained variation in the gut microbiota were determined with forward selection (999
291 Monte Carlo permutations; $P < 0.05$) and used in CCA. CCA analyses were performed in PAST
292 (version 3.01) as previously described (Hazard et al., 2013).

293

294

295 **Acknowledgments**

296 This work was supported by the NERC Centre for Ecology & Hydrology (CEH). We thank Sarah
297 Hulmes, Lucy Hulmes, Jo Savage, and Claire Carvell (all CEH) for field sample collections.

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515 **Figure and Table legends**

516 **Figure 1** Comparisons of community characteristics between bee groups. (a) Distribution and
517 abundance of OTUs from bee gut microbiota samples. Given is the number of samples for which
518 each bacterial taxon was observed to occupy, plotted against the mean abundance across all
519 samples ($n = 23$, $r^2 = 0.68$, $F_{1, 371} = 787.6$, $P < 0.0001$). Common OTUs were defined as those that
520 fell within the upper quartile (dashed lines), and rare OTUs defined as those that did not. (b) Mean
521 OTU richness of whole, common and rare microbiota within the reared restricted (RR), reared foraged
522 (RF) and wild (W) bee groups. Asterisks denote significant differences in comparisons of diversity at
523 the $P < 0.05$ level determined by two-sample t -tests (t -test summary statistics are given in Table S1).
524 (c) Taxa turnover within whole (solid squares), common (solid circles) and rare (open circles)
525 microbiota between sample groups. (d) Dendrogram of similarity between groups partitioned into the
526 whole (W), common (C) and rare (R) microbiota. Metacommunity profiles were compared using the
527 Bray-Curtis quantitative index of similarity and unweighted pair-group method using arithmetic mean
528 (UPGMA).

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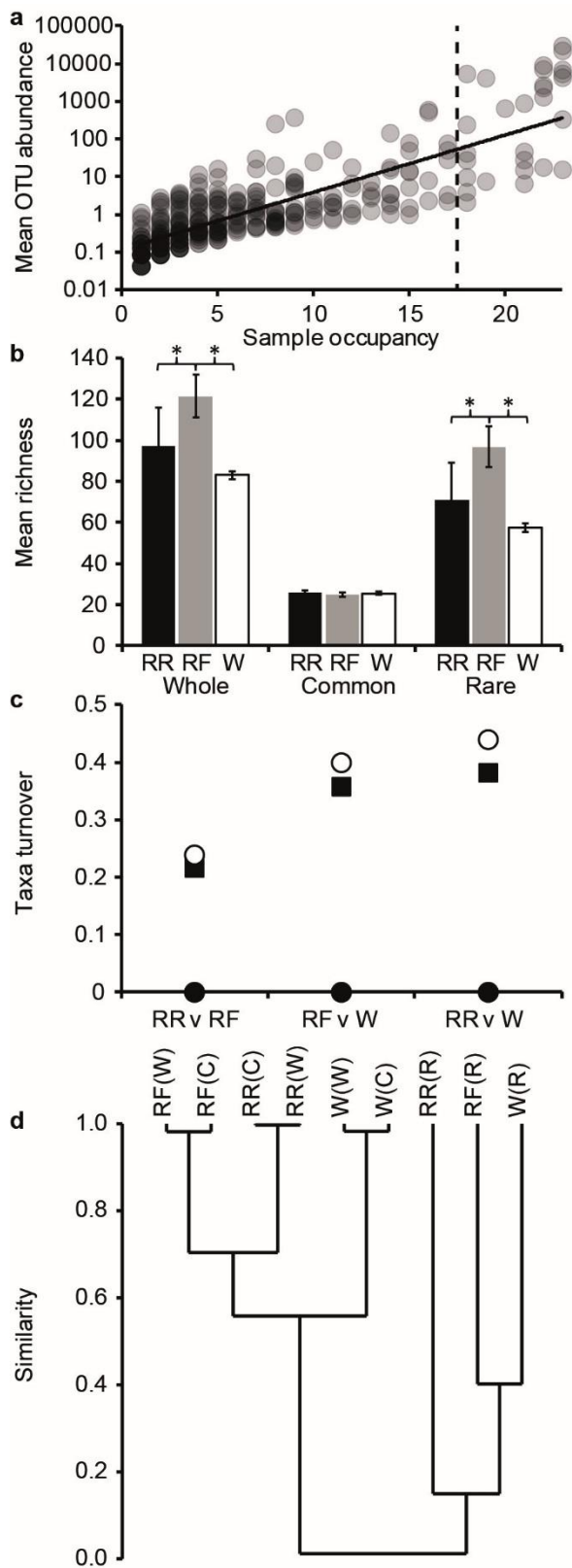
530 **Figure 2** Unique and shared OTUs between groups. Values given within circles represent, unique
531 OTUs to the reared restricted (RR) group, reared foraged (RF), and wild (W) groups. Values given in
532 overlapping regions correspond to the number of OTUs shared between two given groups. Central
533 overlapping region corresponds to OTUs shared across all group types inclusive of the 28 common
534 OTUs. The arrow represents direction of proposed community transition from commercially reared to
535 wild type microbiota.

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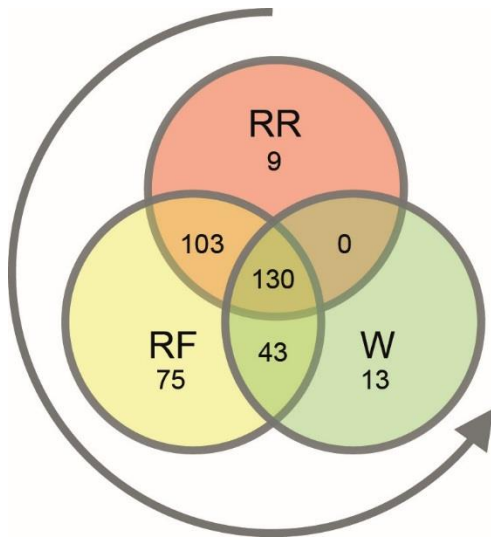
539 **Figure 1**



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542 **Figure 2**



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544 **Table 1** Analysis of similarity (ANOSIM) of whole, common, and rare microbiota between reared
 545 restricted (RR), reared foraged (RF), and wild (W) bee groups. ANOSIM test statistic (*R*) and
 546 probability (*P*) that two compared groups are significantly different at the $P < 0.05$ level (denoted with
 547 asterisks) are given in the lower and upper triangles, respectively. ANOSIM *R* and *P* values were
 548 generated using the Bray-Curtis measure of similarity.

549
 550

Whole	RR	RF	W
RR	-	0.990	0.008*
RF	-0.177	-	0.832
W	0.295	-0.085	-
Common	RR	RF	W
RR	-	0.992	0.009*
RF	-0.177	-	0.869
W	0.298	-0.092	-
Rare	RR	RF	W
RR	-	0.107	0.01*
RF	0.219	-	0.266
W	0.664	0.129	-

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554 **Table 2** Similarity of percentages (SIMPER) analysis of bacterial community dissimilarity (Bray-Curtis) between Reared Restricted (RR), Reared Foraged (RF), and
 555 Wild (W) sample group whole microbiota. Given is mean % abundance of sequences for operational taxonomic units across the samples each was observed to
 556 occupy and the average dissimilarity between samples ((RR vs. RF) = 58% and (RR vs. W) = 59%, (RF vs. W) = 67%). Percentage contribution is the mean
 557 contribution divided by mean dissimilarity across samples. The list of OTUs is not exhaustive so cumulative % value does not sum to 100%. All OTUs listed belong
 558 to the common microbiota. Given the length of the ribosomal sequences analyzed, OTU identities should be considered putative.

559

Class	Family	Taxon name	% Mean abundance				Cont%	Cuml. %
			RR	RF	W	Av. dis.		
Betaproteobacteria	Neisseriaceae	<i>Snodgrassella alvi</i> 99%	52.1	29.5	22.4	16.16	26.19	26.19
Gammaproteobacteria	Orbaceae	<i>Gilliamella apicola</i> 99%	22.3	17.9	30.9	9.50	15.38	41.57
Gammaproteobacteria		Enterobacteriaceae	<i>Arsenophonus nasoniae</i> 99%	0.02	6.06	15.8	6.84	11.08
Flavobacteriia	Flavobacteriaceae	<i>Flavobacterium</i> 83%	0.00	9.31	7.76	5.39	8.74	61.39
Bacilli	Lactobacillaceae	<i>Lactobacillus</i> 91%	6.72	7.70	1.84	4.31	6.98	68.37
Bacilli	Leuconostocaceae	<i>Fructobacillus</i> 100%	0.02	0.29	12.5	4.18	6.78	75.15
Gammaproteobacteria	Enterobacteriaceae	<i>Yokenella</i> 98%	7.44	6.04	0.25	4.03	6.52	81.67
Bacilli	Lactobacillaceae	<i>Lactobacillus kunkeei</i> 100%	0.17	4.63	3.78	2.86	4.63	86.31
Bacilli	Enterococcaceae	<i>Vagococcus</i> 100%	4.47	3.66	0.05	2.50	4.04	90.35
Gammaproteobacteria	Streptococcaceae	<i>Lactococcus</i> 98%	0.12	4.92	0.06	1.92	3.11	93.46
Gammaproteobacteria	Pseudomonadaceae	<i>Pseudomonas</i> 100%	2.66	2.27	0.01	1.53	2.48	95.93
Actinobacteria	Bifidobacteriaceae	<i>Bombiscardovia coagulans</i> 98%	1.06	1.73	0.87	0.94	1.52	97.46
Bacilli	Enterococcaceae	<i>Enterococcus</i> 100%	0.98	1.29	0.04	0.68	1.11	98.56

560

561 **Table 3** Canonical correspondence analyses (CCA) for determination of percent variation in the whole,
562 common, and rare microbiota between the three subject groups by environmental variables significant at the
563 $P < 0.05$ level. CCA biplots are given in Figure S1.

564

	Whole		Common		Rare	
	% variance	<i>P</i>	% variance	<i>P</i>	% variance	<i>P</i>
Foraging	8.44	0.001	8.45	0.001	6.55	0.001
Rearing	7.93	0.002	7.93	0.001	10.74	0.001
Host weight	3.10	0.002	2.76	0.001	10.25	0.001
Undetermined	80.53	-	80.86	-	72.46	-

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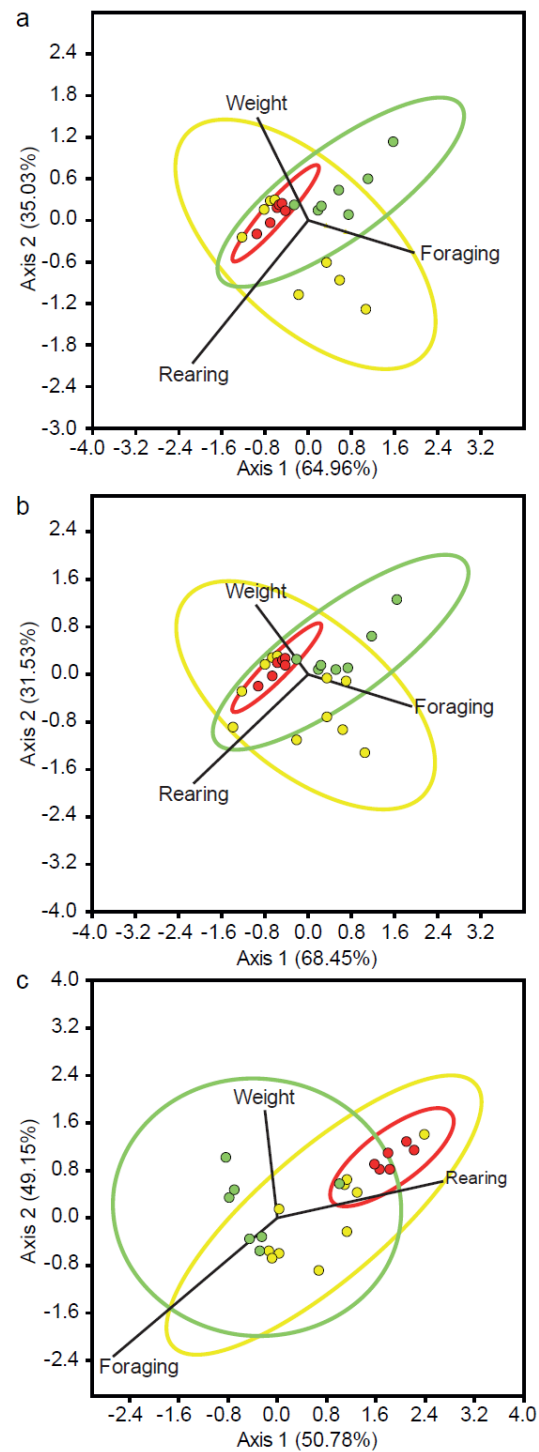


Figure S1 Canonical correspondence bi-plots for (a) whole, (b) common, and (c) rare microbiota. Solid red circles represent microbiota samples from the reared restricted (RR) group, solid yellow circles for the reared foraged (RF) group, and solid green circles for the wild (W) group. In each instance, the 95 % concentration ellipses are given for the RR (red), RF (yellow), and W (green) group microbiota. Bi-plot lines for variables that significantly accounted for variation within the microbiota at the $P < 0.05$ level (see Table 3) show the direction of increase for each variable, and the length of each line indicates the degree of correlation with the ordination axes. CCA field labels: rearing, foraging, and host weight. Percentage of community variation explained by each axis is given in parentheses.

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570 **Table S1** Two-sample *t*-tests comparing mean whole, common, and rare microbiota richness between
 571 reared restricted (RR), reared foraged (RF), and wild (W) bee cohorts. Two-sample *t*-test statistic (*t*) and
 572 significance (*P*) that richness between two compared groups is significantly different at the *P* < 0.05 level
 573 (denoted with asterisks) are given in the lower and upper triangles, respectively.
 574

Whole	RR	RF	W
RR	-	0.027*	0.125
RF	2.91	-	0.0001*
W	1.85	11.45	-
Common	RR	RF	W
RR	-	0.054	0.499
RF	2.18	-	0.07
W	0.71	1.96	-
Rare	RR	RF	W
RR	-	0.019*	0.122
RF	3.2	-	0.0001*
W	1.86	12.33	-

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579 **Table S2** Sample details and barcodes used with their associated samples are given below.

Sample	Origin	Foraged (F) or Restricted (R)	Geography	Total wet weight (g)	Gut wet weight (g)	Barcode Sequence
RR1	Commercially reared	R	n/a	0.175	0.01	GATCTGCGATCC
RR2	Commercially reared	R	n/a	0.194	0.017	AGTCGTGCACAT
RR3	Commercially reared	R	n/a	0.166	0.012	CGAGGGAAAGTC
RR4	Commercially reared	R	n/a	0.233	0.06	CAAATTCGGAT
RR5	Commercially reared	R	n/a	0.207	0.017	AGATTGACCAAC AGTTTACGAGCT
RR6	Commercially reared	R	n/a	0.1512	0.016	A
RF1	Commercially reared	F	Wallingford	0.251	0.04	CAGCTCATCAGC
RF2	Commercially reared	F	Wallingford	0.308	0.036	CAAACAACAGCT
RF3	Commercially reared	F	Wallingford	0.277	0.042	GCAACACCATCC
RF4	Commercially reared	F	Wallingford	0.176	0.034	GCGATATATCGC
RF5	Commercially reared	F	Wallingford	0.192	0.032	GTATCTGCGCGT
RF6	Commercially reared	F	Wallingford	0.15	0.034	GCATATGCACTG
RF7	Commercially reared	F	Wallingford	0.148	0.028	CAACTCCCGTGA
RF8	Commercially reared	F	Wallingford	0.172	0.01	TTGCGTTAGCAG
RF9	Commercially reared	F	Wallingford	0.143	0.014	TACGAGCCCTAA
RF10	Commercially reared	F	Wallingford	0.286	0.025	ATCACCAGGTGT
W1	Wild	F	Hillesden	0.187	0.017	CGAGCAATCCTA
W2	Wild	F	Hillesden	0.196	0.011	TAATACGGATCG
W3	Wild	F	Hillesden	0.261	0.029	CATTCTGGCGT
W4	Wild	F	Isle of Wight	0.339	0.03	TCCCTTGCTCC
W5	Wild	F	Isle of Wight	0.162	0.026	ACGAGACTGATT
W6	Wild	F	Isle of Wight	0.22	0.022	GCTGTACGGATT
W7	Wild	F	Hillesden	0.279	0.03	TGTGAATTCGGA

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